Clinical Utility of YKL-40 in Polymyositis/dermatomyositis-associated Interstitial Lung Disease

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ABSTRACT. ***Objective.*** Interstitial lung disease (ILD) is involved in polymyositis/dermatomyositis (PM/DM), a disease associated with poor prognoses. Chitinase-3-like-1 protein (YKL-40) has pleiotropic biological activities involved in inflammation, cell proliferation, and tissue remodeling; however, the clinical application of YKL-40 remains limited. We investigated the clinical significance of YKL-40 in PM/DM–ILD.

Methods. Sixty-nine consecutive patients with PM/DM–ILD and 34 healthy controls were analyzed. We measured baseline and followup serum YKL-40 using an ELISA, evaluated the association of YKL-40 with clinical variables and survival, and examined YKL-40 expression in lung specimens from patients with PM/DM–ILD using immunohistochemistry.

Results. Serum YKL-40 levels were significantly greater in patients with PM/DM–ILD compared with healthy controls ($p < 0.0001$). Serum YKL-40 was correlated with arterial oxygen pressure ($r = –0.40$, $p < 0.001$) and percent-predicted DLCO ($r = –0.41$, $p = 0.01$) in patients with PM/DM–ILD. Multivariate Cox hazard analysis demonstrated that higher serum YKL-40 and lower percent-predicted forced vital capacity were independently associated with a poor prognosis. Immunohistochemistry analysis demonstrated that YKL-40 expression was enhanced in aggregated intraalveolar macrophages and hyperproliferative alveolar epithelial cells in patients with PM/DM–ILD.

Conclusion. YKL-40 is a promising biomarker for evaluating PM/DM–ILD activity/severity and predicting disease prognosis. Insights into YKL-40 might help elucidate the pathogenesis of PM/DM–ILD. (First Release July 15 2017; J Rheumatol 2017;44:1394–401; doi:10.3899/jrheum.170373)

Key Indexing Terms:
CHITINASE-3-LIKE-1 PROTEIN POLYMYOSITIS DERMATOMYOSITIS
INTERSTITIAL PNEUMONIA INTERSTITIAL LUNG DISEASE

Polymyositis (PM) and dermatomyositis (DM) are systemic inflammatory disorders that involve skeletal muscle, skin, and/or other organs[1,2,3]. Patients with PM/DM frequently present with interstitial lung disease (ILD). Although the clinical course of ILD varies greatly, it is a major cause of morbidity and mortality, with an estimated excess mortality rate of 40%[4,5,6,7,8,9,10,11,12]. Therefore, early diagnosis, accurate classification, and careful monitoring are essential for effectively managing patients with PM/DM-associated ILD. Studies have demonstrated that serum myositis-specific

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Supported by a grant from the Japan Society for the Promotion of Science (JP16K19449 to H.H.).

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Accepted for publication May 13, 2017.

autoantibodies (MSA) are valuable serum biomarkers for diagnosing PM/DM–ILD and classifying patients into distinct subgroups[12–21,22]. However, no serum biomarker for evaluating disease activity/severity and predicting outcomes in patients with PM/DM–ILD has yet been established; it remains an unmet need in clinical practice.

YKL-40, also referred to as chitinase-3-like-1 and human cartilage glycoprotein 39, is a glycoprotein primarily secreted by macrophages, neutrophils, and certain types of local epithelial cells. YKL-40 belongs to the chitinase family of evolutionarily conserved hydrolases that are characterized by their ability to cleave the polysaccharide chitin[23,24,25,26]. The precise physiological function of YKL-40 remains unclear; however, previous reports have implicated YKL-40 in inflammation, cell proliferation, and tissue remodeling[23,24,25,26]. Previous studies have also investigated the relationship between YKL-40 and several human diseases, including idiopathic pulmonary fibrosis (IPF)[27,28,29,30,31,32,33]. We demonstrated previously that serum YKL-40 level was associated with impaired oxygenation and that YKL-40 was expressed in the lungs of patients with IPF[30], suggesting that YKL-40 might be involved in the pathogenesis of ILD characterized by abnormal tissue remodeling and subsequent fibrosis. It could also be a useful biomarker in other ILD as well as IPF. However, the clinical significance of YKL-40 in PM/DM–ILD is yet to be determined. Therefore, we evaluated the association of serum YKL-40 level with clinical variables and survival, and examined YKL-40 expression in lung specimens from patients with PM/DM–ILD using immunohistochemistry.

MATERIALS AND METHODS

Subjects. We evaluated 72 consecutive patients diagnosed with PM/DM–ILD between 1996 and 2016 at the Hamamatsu University Hospital (Hamamatsu, Japan) and 34 healthy controls (HC) and conducted a retrospective review of their clinical records. The study was conducted in accordance with the Declaration of Helsinki. Signed consent forms were obtained from all study participants with the exception of those who died before September 2016. The institutional review board of the Hamamatsu University School of Medicine waived the informed consent requirement for deceased patients and approved the study (approval number E15-062).

Our present study included ILD patients with definite or probable PM/DM or clinically amyopathic DM (CADM) and HC. Definite or probable PM/DM was diagnosed according to Bohan and Peter's criteria[1,2]. CADM was diagnosed if the patient had a typical rash with little or no clinical evidence of myopathy during the study period[3,9,10,11,12,13,14]. Almost all of the patients underwent a systemic examination for malignancies at the time of PM/DM–ILD diagnosis. ILD was diagnosed on the basis of clinical, high-resolution computed tomography, with/without lung biopsy findings. ILD onset was classified as acute (worsening < 1 mo from the onset of respiratory symptoms or the initial visit), subacute (worsening within 1–3 mos), or chronic (stable or slowly progressive > 3 mos).

Clinical data at the time of diagnosis, including patient characteristics, laboratory data, and pulmonary function, were obtained from patient medical records.

Measurement of serum YKL-40 levels and detection of MSA. Baseline serum samples collected at the time of diagnosis were available for 69 of the 72 patients with PM/DM–ILD. The remaining 3 patients were excluded from the study. Followup serum samples obtained 1 and 6 months after the initiation of PM/DM–ILD treatment were available for 28 and 27 of the 69 patients, respectively. Serum samples were also collected from 34 HC. The samples were stored at –20°C or –80°C until further analysis. Serum YKL-40 levels were determined using a YKL-40 ELISA (CircuLex Human YKL-40 ELISA Kit; MBL) according to the manufacturer's instructions. The presence of MSA, including antiaminoacyl tRNA-synthetase (ARS; e.g., anti-PL-7, -Jo1, -PL-12, -KS, -EJ, -OJ), antimelanoma differentiation-associated gene 5 (MDA5), antisignal recognition particle, anti-transcriptional intermediary factor 1-γ, and other antibodies, were measured using RNA immunoprecipitation (IP) and protein IP assays[14,15,16].

YKL-40 immunohistochemistry. Formalin-fixed, paraffin-embedded sections (5-μm thickness) of surgically resected lung biopsy specimens from patients with PM/DM–ILD were analyzed using immunohistochemistry. The sections were deparaffinized and preheated for 30 min in epitope retrieval solution (Dako Target Retrieval Solution S1700; Agilent). Goat anti-human YKL-40 (10 μg/ml) was used as the primary antibody (Human Chitinase 3-like 1 Antibody; R&D Systems Inc.). The samples were blocked and stained using the Anti-Goat HRP-DAB Cell and Tissue Staining Kit (R&D Systems Inc.) according to the manufacturer's instructions. The immunoreaction was visualized using 3, 3-diaminobenzidine chromogen and the sections were counterstained with hematoxylin. Normal lung tissues obtained from sites distant from the lesion in patients with lung cancer who had never smoked and did not have asthma were used as controls.

Statistical analysis. All values are expressed as the median (range) or number (%). The observation period was calculated from the date of diagnosis until the last visit or time of death. Mann–Whitney U, Fisher's exact, and Wilcoxon signed-rank tests were used to compare continuous variables, proportions, and paired data, respectively. Multiple pairwise comparisons with Bonferroni correction were used to adjust p values. The correlations between serum YKL-40 levels and clinical variables were analyzed using the Spearman correlation coefficient. Receiver-operating characteristics (ROC) analysis was used to identify the optimal cutoff level with which to use serum YKL-40 as a prognostic marker. Overall survival was evaluated using the Kaplan–Meier method and survival curves were compared using the log-rank test. Cox hazards analysis was used to identify variables associated with survival. $P < 0.05$ was considered statistically significant. All of the data were analyzed using JMP software version 9.0.3a (SAS Institute Inc.) and R software version 2.15.1 (The R Foundation for Statistical Computing).

RESULTS

Baseline characteristics. Baseline characteristics are summarized in Table 1. The median age of patients with PM/DM–ILD was 53 years, and 45 (65.2%) of the 69 patients were women. Three patients were diagnosed with an early stage malignancy (breast cancer, n = 2 and stomach cancer, n = 1) at the time of PM/DM–ILD diagnosis. However, the malignancies did not affect PM/DM–ILD prognosis because they were completely resected and subsequently cured. Eighteen (26.1%) of the 69 patients with PM/DM–ILD died during the study period. Thirteen deaths were because of PM/DM–ILD, including respiratory failure (n = 12) and infection (n = 1), 4 were attributed to malignancies that developed after PM/DM–ILD diagnosis (cancer of unknown primary origin, n = 1, pharyngeal cancer, n = 2, and lung cancer, n = 1), and 1 was attributed to the rupture of an abdominal aortic aneurysm.

The initial treatment regimens are presented in Supplementary Table 1 (available with the online version of this article). Among the 69 patients, 21 (30.4%) were treated with

Table 1. Baseline characteristics (patients with PM/DM-ILD, n = 69). Values are median (range) or n (%).

Characteristics	Values
Age, yrs	53 (32–77)
Male/female	24 (34.8)/45 (65.2)
Smoking status, never/former/current	40 (58.0)/15 (21.7)/14 (20.3)
ILD onset, chronic/subacute/acute	33 (47.8)/22 (31.9)/14 (20.3)
Myositis diagnosis, DM/CADM/PM	33 (47.8)/29 (42.0)/7 (10.1)
MSA, ARS/MDA5/SRP/TIF1-γ/negative	29 (42.0)/17 (24.6)/3 (4.3)/1 (1.4)/19 (27.5)
Malignancy at the time of diagnosis	3 (4.3)*
Laboratory data	
CPK, IU/l	179 (24–8820)
Ferritin, ng/ml	137 (14–12,701)
KL-6, U/ml	772 (224–6192)
SP-D, ng/ml	108 (17–1090)
PaO_2, Torr	75 (48–109)
Pulmonary function	
%FVC	69 (38–113)
$FEV_{1.0}$/FVC, %	83 (68–100)
%DLCO	71 (27–137)
Observation period, yrs	5.2 (0.2–18.8)
Death during observation period	18 (26.1)

* Breast cancer (n = 2), stomach cancer (n = 1). PM: polymyositis; DM: dermatomyositis; ILD: interstitial lung disease; CADM: clinically amyopathic dermatomyositis; MSA: myositis-specific autoantibody; ARS: antiaminoacyl tRNA-synthetase antibody; MDA5: antimelanoma differentiation-associated gene 5 antibody; SRP: antisignal recognition particle antibody; TIF1-γ: antitranscriptional intermediary factor 1-γ antibody; CPK: creatine phosphokinase; KL-6: Krebs von den Lungen-6; SP-D: surfactant protein-D; PaO_2: arterial oxygen pressure; Torr: a unit of pressure based on an absolute scale, defined as exactly 1/760 of a standard atmosphere; %FVC: predicted forced vital capacity; $FEV_{1.0}$: forced expiratory volume 1.0 s.

prednisolone alone and 47 (68.1%) were treated with prednisolone and immunosuppressants (cyclosporine, n = 30, tacrolimus, n = 8, intravenous cyclophosphamide, n = 2, azathioprine, n = 1, intravenous cyclophosphamide plus cyclosporine, n = 5, and intravenous cyclophosphamide plus tacrolimus, n = 1). One patient (1.5%) was stable without treatment during the study period.

Serum YKL-40 levels. There was a significant difference in baseline serum YKL-40 levels between patients with PM/DM–ILD and HC (Supplementary Figure 1, available with the online version of this article). The median age (range) of the HC was 53 years (27–71), and 19 (55.9%) of the 34 HC were women. There were no significant differences in age (p = 0.54) or sex (p = 0.39) between patients with PM/DM–ILD and HC. The median serum YKL-40 level (range) was significantly greater in patients with PM/DM–ILD compared to HC (56.7, 1.3–233 vs 26.4, 2.4–69.4 ng/ml, respectively; p < 0.0001).

Median serum YKL-40 levels were 43.7, 61.3, and 100.9 ng/ml in PM/DM–ILD patients with chronic, subacute, and acute-onset ILD, respectively (p = 0.03; Supplementary Figure 2, available with the online version of this article), and 42.9, 82.3, and 61.6 ng/ml in PM/DM–ILD patients with CADM, DM, and PM-ILD, respectively (p = 0.02; Supplementary Figure 3, available with the online version of this article). In contrast, there were no significant differences in serum YKL-40 levels among smoking status (Supplementary Figure 4, available with the online version of this article) or MSA subgroups (Supplementary Figure 5, available with the online version of this article).

Correlation between serum YKL-40 and clinical variables. The correlations between serum YKL-40 levels and clinical variables in patients with PM/DM–ILD are presented in Table 2. Serum YKL-40 levels demonstrated a significant positive correlation with age, serum Krebs von den Lungen-6 (KL-6)

Table 2. Correlation between serum YKL-40 level and clinical variables.

Characteristics	r	p
Age, yrs	0.41	< 0.001*
Laboratory data		
KL-6, U/ml	0.33	< 0.01*
SP-D, ng/ml	–0.10	0.40
CPK, U/ml	0.15	0.21
Ferritin, ng/ml	0.46	< 0.001*
PaO_2, Torr	–0.40	< 0.001*
Pulmonary function		
%FVC	–0.18	0.16
$FEV_{1.0}$/FVC, %	–0.04	0.76
%DLCO	–0.41	0.01*

* p < 0.05. KL-6: Krebs von den Lungen-6; SP-D: surfactant protein-D; CPK: creatine phosphokinase; PaO_2: arterial oxygen pressure; Torr: a unit of pressure based on an absolute scale, defined as exactly 1/760 of a standard atmosphere; %FVC: predicted forced vital capacity; $FEV_{1.0}$: forced expiratory volume 1.0 s.

levels, and serum ferritin levels, and a significant negative correlation with arterial oxygen pressure (PaO_2) and percent DLCO, but not with percent forced vital capacity (FVC).

Changes in serum biomarker levels are presented in Figure 1. In patients who survived the first 90 days of the study (Survivors), there was a significant reduction in YKL-40 levels 1 month after treatment initiation (28 ng/ml) compared with baseline levels (59 ng/ml, $p < 0.01$). In contrast, serum KL-6 levels 1 month after treatment initiation (1070 U/ml) did not decrease from baseline levels (803 U/ml, $p = 0.90$). However, serum KL-6 levels significantly decreased from baseline 6 months after treatment initiation (499 U/ml, $p < 0.01$). Notably, serum YKL-40 levels decreased much earlier than serum KL-6 levels in patients who responded to treatment.

YKL-40 expression in lung biopsy tissue specimens. We examined YKL-40 expression in lung tissues obtained from patients with PM/DM–ILD (n = 7) and controls (n = 3) using immunohistochemistry (Figure 2). Control lungs exhibited a small number of YKL-40–positive alveolar macrophages in the intraalveolar space and faint YKL-40 expression in bronchial epithelial cells. In contrast, we consistently observed aggregates of YKL-40–positive alveolar macrophages, hyperproliferative YKL-40–positive alveolar epithelial cells adjacent to fibrotic lesions, and YKL-40 expression in bronchial epithelial cells in lung tissues from patients with PM/DM–ILD. YKL-40 was not expressed in any fibrotic lesions, including fibroblastic foci. These observations suggest that aggregates of intraalveolar macrophages and hyperproliferative alveolar epithelial cells, but not fibrotic tissues, are potential sources of YKL-40 in patients with PM/DM–ILD.

Survival analysis. The univariate Cox hazard analysis (Table 3) revealed that seropositive status for anti-ARS antibodies, higher PaO_2 levels, and higher %FVC were associated with longer survival. In contrast, seropositive status for anti-MDA5 antibodies, acute/subacute-onset ILD, and higher serum ferritin levels were associated with shorter survival time. Further, higher serum YKL-40 levels were associated with poorer prognoses. Because anti-ARS and anti-MDA5 antibodies are mutually exclusive[12,15,20], we adjusted for each of these factors in separate multivariate analyses of each clinical variable identified as significant in the univariate analysis (Table 3). In both multivariate analyses, higher serum YKL-40 level and lower %FVC were independently associated with a poorer prognosis.

The ROC curve of serum YKL-40 levels and death within 2 years from diagnosis is presented in Supplementary Figure 6 (available with the online version of this article). Using the optimal cutoff level of serum YKL-40 (105 ng/ml), the area under the curve was 0.78, and the sensitivity and specificity were 75.0% and 82.6%, respectively. Patients with PM/DM–ILD were classified as YKL-40^{high} and YKL-40^{low} based on the optimal cutoff level. Kaplan–Meier survival curves (Figure 3) demonstrated that the YKL-40^{high} group had a significantly lower 5-year survival rate compared with the YKL-40^{low} group (41.6% vs 93.6%, $p < 0.001$).

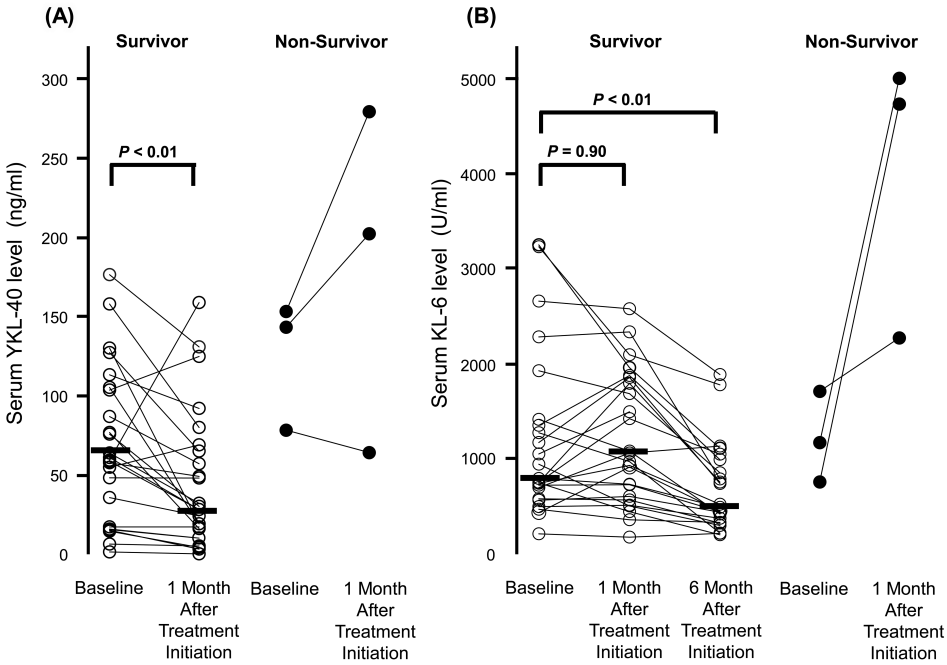

Figure 1. Changes in serum biomarkers. (A) Serum YKL-40 levels significantly decreased from baseline 1 month after treatment initiation in patients with PM/DM–ILD who survived the initial 90 days of the study (Survivors). (B) Serum KL-6 levels did not decrease from baseline 1 month after treatment initiation, although KL-6 levels eventually decreased from baseline 6 months after treatment initiation. PM: polymyositis; DM: dermatomyositis; ILD: interstitial lung disease; KL-6: Krebs von den Lungen-6; YKL-40: chitinase-3-like-1 protein.

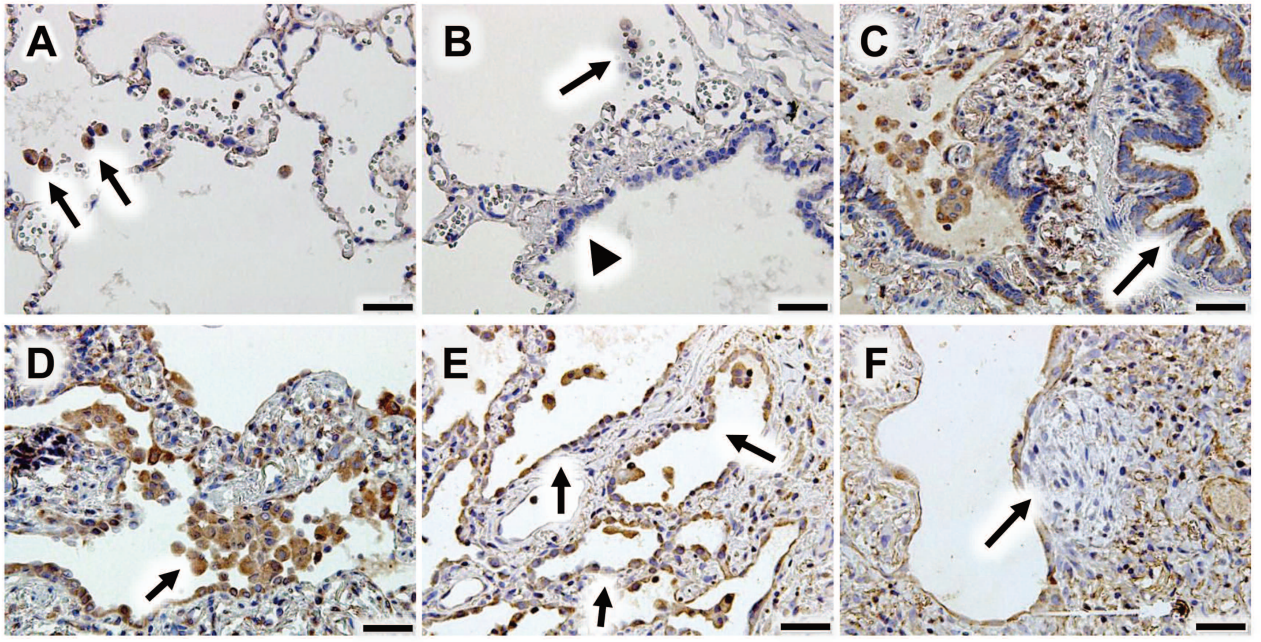

Figure 2. YKL-40 immunostaining in lung specimens from controls and patients with PM/DM–ILD. Control lungs (× 200 magnification, scale bar: 20 μm): (A) A small number of YKL-40–positive macrophages were observed in the intraalveolar space (arrows). (B) YKL-40–positive macrophages (arrow) and faint YKL-40 expression in bronchial epithelial cells (arrowhead). Lung tissues from patients with PM/DM (×200 magnification, scale bar: 20 μm): (C) YKL-40 was strongly expressed in bronchial epithelial cells (arrow). (D) Dense aggregates of YKL-40–positive alveolar macrophages (arrow). (E) Hyperproliferative YKL-40–positive alveolar epithelial cells adjacent to fibrotic lesions (arrows). (F) YKL-40 expression was not observed in any fibrotic lesions, including fibroblastic foci (arrow). PM: polymyositis; DM: dermatomyositis; ILD: interstitial lung disease; YKL-40: chitinase-3-like-1 protein.

DISCUSSION

Our current study demonstrated that serum YKL-40 levels were significantly greater in patients with PM/DM–ILD compared with HC and that serum YKL-40 was correlated with PaO_2 and %DLCO in patients with PM/DM–ILD. YKL-40 expression increased in the lungs of patients with PM/DM–ILD compared with control lungs, and higher serum YKL-40 level was independently associated with a poorer prognosis. To our knowledge, ours is the first study to identify the clinical significance of YKL-40 in patients with PM/DM–ILD.

Although YKL-40 induction has been demonstrated in several human diseases[27,28,29,30,31,32,33], no study has investigated the clinical and pathophysiological roles of YKL-40 in PM/DM patients with or without ILD. YKL-40 is expressed by various cell types, including macrophages, neutrophils, synovial cells, chondrocytes and bronchial, colonic, and ductal epithelial cells. YKL-40 expression is regulated by various cytokines, including interleukin (IL)-6, IL-13, interferon-γ, tumor necrosis factor-α, and IL-1β, that mediate the production of inflammatory mediators, cell proliferation, and tissue remodeling, thereby promoting fibrogenesis through transforming growth factor-β induction[25,26,34]. Because PM and DM are groups of a systemic autoimmune inflammatory disorder, elevated serum YKL-40 might be derived from systemic inflammatory cells or local cells in blood, muscle, skin, lung, or other organs. Indeed, previous studies reported that YKL-40 production can be mediated by affected organs in several other diseases[25,26,29,30,35,36]. Our present study also demonstrated that serum YKL-40 is correlated with oxygenation impairment in patients with PM/DM–ILD. Further, YKL-40 expression was enhanced in aggregated intraalveolar macrophages, bronchial epithelial cells, and proliferative alveolar epithelial cells. Ours is the first report of YKL-40 expression in hyperproliferative alveolar epithelial cells in patients with lung disease. These data suggest that lung tissue is a key source of YKL-40 and plays a pathophysiological role in PM/DM–ILD.

Serum YKL-40 was an independent prognostic factor in PM/DM–ILD. Among patients who died during the study period, the most common cause of death was ILD-associated respiratory failure. The correlation between serum YKL-40 levels and oxygenation impairment suggested that high levels of serum YKL-40 directly reflected ILD severity in patients with PM/DM–ILD. However, a small number of patients died because of a malignancy that developed after PM/DM–ILD diagnosis. YKL-40 has also been implicated in cancer cell proliferation, invasiveness, and angiogenesis[37]. In our present study, baseline serum YKL-40 levels in patients who died

Table 3. Cox proportional hazards regression analysis of mortality.

Characteristics	HR	95% CI	p
Univariate analysis			
Anti-ARS antibody–positive	0.57	0.29–0.96	0.035*
Anti-MDA5 antibody–positive	1.80	1.08–2.92	0.025*
Female	0.67	0.41–1.09	0.11
Age, yrs	1.04	0.99–1.09	0.09
Smoking, current/former, vs never	1.15	0.70–1.86	0.57
ILD onset, acute/subacute, vs chronic	2.11	1.24–3.99	< 0.01*
Myositis diagnosis, CADM/DM, vs PM	0.81	0.46–1.70	0.53
PaO_2, per 10 Torr increase	0.49	0.33–0.74	< 0.001*
%FVC, per 10% increase	0.62	0.42–0.87	< 0.01*
$FEV_{1.0}$/FVC, per 10% increase		0.55	0.25–1.17 0.12
KL-6, per 100 U/ml increase	1.01	0.95–1.05	0.71
SP-D, per 10 ng/ml increase	0.99	0.95–1.02	0.54
CPK, per 100 U/ml increase	1.004	0.96–1.03	0.84
Ferritin, per 100 ng/ml increase	1.03	1.01–1.04	< 0.01 *
YKL-40, per 10 ng/ml increase	1.15	1.07–1.24	< 0.001*
Multivariate analysis			
Adjusted for anti-ARS antibody status			
PaO_2, per 10 Torr increase	0.62	0.32–1.18	0.15
%FVC, per 10% increase	0.64	0.42–0.90	< 0.01*
Ferritin, per 100 ng/ml increase	1.02	0.99–1.04	0.15
YKL-40, per 10 ng/ml increase	1.15	1.04–1.28	< 0.01*
Adjusted for anti-MDA5 antibody status			
PaO_2, per 10 Torr increase	0.61	0.31–1.16	0.13
%FVC, per 10% increase	0.65	0.42–0.93	0.01*
Ferritin, per 100 ng/ml increase	1.02	0.99–1.04	0.20
YKL-40, per 10 ng/ml increase	1.15	1.04–1.29	< 0.01*

* $p < 0.05$. ARS: antiaminoacyl tRNA-synthetase antibody; MDA5: antimelanoma differentiation-associated gene 5 antibody; ILD: interstitial lung disease; CADM: clinically amyopathic dermatomyositis; DM: dermatomyositis; PM: polymyositis; PaO_2: arterial oxygen pressure; Torr: a unit of pressure based on an absolute scale, defined as exactly 1/760 of a standard atmosphere; %FVC: predicted forced vital capacity; $FEV_{1.0}$: forced expiratory volume 1.0 s; KL-6: Krebs von den Lungen-6; SP-D: surfactant protein-D; CPK: creatine phosphokinase; YKL: chitinase-3-like-1 protein.

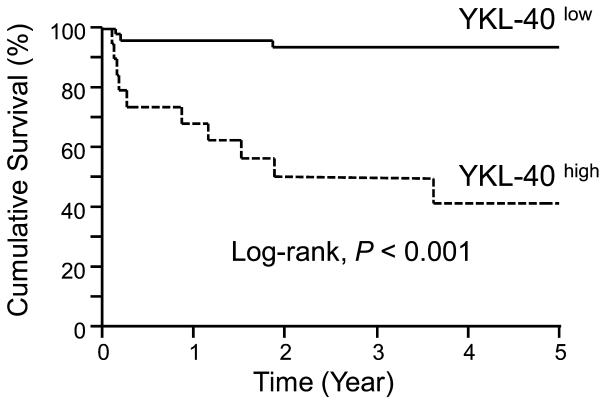

Figure 3. Kaplan–Meier survival curve. The 5-year survival rate was 93.6% in the YKL-40 low group (serum YKL-40 < 105 ng/ml) and 41.6% in the YKL-40 high group (serum YKL-40 > 105 ng/ml); $p < 0.001$ by log-rank test. YKL-40: chitinase-3-like-1 protein.

from malignancy were high (median 136 ng/ml, range 106–192 ng/ml), even though the malignancy had not yet been diagnosed. These observations suggest that high levels of YKL-40 might induce oncogenesis or promote the proliferation of latent cancer cells. Collectively, the findings of our current study demonstrated that serum YKL-40 levels were correlated with ILD severity, a factor associated with increased mortality. However, in some patients, the increased risk of mortality might have been associated with oncogenesis. Therefore, this issue merits further investigation.

Several studies have investigated potential biomarkers of PM/DM-ILD, including MSA, ferritin, and KL-6[12,13,14,18,38,39,40]. Several MSA, including anti-ARS and anti-MDA5, are useful for classifying PM/DM–ILD into distinct subtypes[12–21,22]; however, the association between MSA titers and disease activity/severity remains unknown. High serum ferritin is associated with an increased risk of death in anti-MDA5–positive patients[18]. KL-6 is a serum biomarker used to monitor several types of ILD[41]. Although serum KL-6 levels are correlated with pulmonary function[38], its prognostic value in PM/DM–ILD has not been established. We also found that serum YKL-40 levels decreased earlier than serum KL-6 levels in patients who responded to treatment. Most importantly, serum YKL-40 levels were correlated with oxygenation impairment and independently

associated with mortality, regardless of the MSA status. These findings indicate that serum YKL-40 is a valuable marker of PM/DM–ILD in the clinical setting.

Previous studies reported that lung tissue is a potential source of YKL-40 in patients with asthma[29] or IPF[30]. In patients with asthma, YKL-40 expression in the bronchial epithelium promotes the thickening of the subepithelial basement membrane, a feature associated with airway flow limitation and disease severity[29]. In patients with IPF, YKL-40 expression is enhanced in aggregated intraalveolar macrophages and bronchial epithelial cells adjacent to fibrotic lesions, and serum YKL-40 levels are correlated with oxygenation impairment[30]. Those observations suggest that YKL-40 plays a distinct pathophysiological role in different diseases. Interestingly, YKL-40 was expressed in the lung and correlated with oxygenation impairment in patients with PM/DM–ILD, consistent with observations in patients with IPF. However, IPF and PM/DM–ILD are distinctly unique ILD. Nordenbaek, *et al* demonstrated that serum YKL-40 levels were elevated in systemic sclerosis (SSc) patients with ILD[33]. These findings suggest that YKL-40 might be involved not only in IPF, PM/DM–ILD, and SSc-ILD, but in other ILD as well. Further studies are needed to confirm this hypothesis.

Our study had several limitations. First, the retrospective design and inclusion of patients with ILD who visited a pulmonary division subjected the study to several potential biases. For example, because our institution is a regional referral center for ILD, referral or selection bias might have led to an enrichment of patients with pulmonary manifestations. In addition, because of the small sample size, the results of the multivariate analyses should be carefully interpreted. Finally, the treatment regimen was not consistent among the study patients. However, most of the patients were treated with corticosteroids in combination with immunosuppressants.

We demonstrated that serum YKL-40 levels were correlated with various clinical variables, including PaO_2 and %DLCO, and were independently associated with mortality. YKL-40 was strongly expressed in intraalveolar macrophages and proliferative alveolar epithelial cells. Together, these findings suggest that YKL-40 is a promising noninvasive biomarker for evaluating disease activity/severity, predicting prognosis, and clarifying the pathogenesis of PM/DM–ILD. Therefore, a prospective, multicenter study is needed to validate the clinical and pathophysiological utility of serum YKL-40 in PM/DM–ILD.

ACKNOWLEDGMENT

The authors thank S. Ibuki from the Department of Rheumatology and Clinical Immunology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, for technical support with immunoprecipitation measurements. The authors also thank M. Hashiguchi from Medical and Biological Laboratories Co. Ltd., Tokyo, Japan, for his assistance.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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